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(54) Title: COMPOSITIONS COMPRISING NATURAL AGENTS FOR TREATMENT OF CANCER

(57) Abstract: Compositions are provided comprising five or more natural agents combined into one formulation, each natural agent possessing known anticancer activity, wherein the natural agents in the composition interact synergistically to enhance their anticancer activities. The compositions of this invention demonstrate *in vitro* inhibition of cancer cell proliferation and provide potential candidates for cancer therapies.

COMPOSITIONS COMPRISING NATURAL AGENTS FOR TREATMENT OF CANCER

BACKGROUND OF THE INVENTION

Field of the Invention:

This invention relates methods of treating cancers comprising administering to a patient a composition comprising five or more natural agents. Compositions comprising four or more natural agents are also provided.

Description of the State of Art:

The concept of using combinations of drugs in cancer therapy is known in the art. Conventional chemotherapy drugs (i.e., synthetic or chemically manufactured drugs) have been used in various combinations (known as "cocktails") since the 1960's. The initial purpose of combination therapy was to increase the effect (i.e., cytotoxicity) of the various chemotherapy drugs and to minimize the development of drug-resistant tumor cells. The argument behind combining chemotherapy drugs was that the combination of drugs would act synergistically, that is, the total effect of the combination of drugs would be greater than the sum of the of the individual effects of each discrete drug. The desired outcome of combining drugs to act synergistically is that the toxicity to cancer cells would remain high, whereas toxicity to the patient would not. However,

most conventional chemotherapy drugs are poisonous to normal cells as well as cancer cells. Thus, the use of these drugs in combination (i.e., in chemotherapy "cocktails") has resulted in limited clinical success, and in some cases has added to the burden of suffering endured by cancer patients, since the toxic side effects of each drug in the chemotherapy "cocktail" are typically additive as well.

Researchers have been actively exploring new avenues of cancer treatment. Recently, there has been much interest in the use of natural

agents as an alternative in the treatment of cancer. The use of natural agents in cancer therapy is attractive in part because natural agents are less toxic to the patient than most chemotherapy drugs. For example, the average of the oral lethal doses (LD_{50}) in rats that is predicted for the preferred natural agents of this invention is 1.5 g/kg. This is in contrast to the 21-fold lower geometric average of 72 mg/kg for a sample of 17 chemotherapy drugs. Further, the most toxic natural compound is far less toxic to normal cells than the most toxic chemotherapy drug. However, these natural agents are also much less toxic to cancer cells than conventional chemotherapy drugs. Therefore, a disadvantage to using individual natural agents in cancer therapy is that significantly higher doses of natural compounds are required to inhibit cancer as compared to conventional chemotherapy drugs. These higher doses are often higher than would be practical for cancer therapy. Moreover, natural compounds tend to have less favorable pharmacokinetics than most drugs, and therefore higher doses are required to reach a given plasma concentration. Consequently, most natural compounds could be expected to product adverse effects if used at a dose that would produce effective plasma concentrations. Therefore, if natural compounds are to be used with a high degree of safety, they must be used at lower doses.

There are a few reports on small combinations of natural agents that suggest that when used in combination, they can produce additive or supra-additive (synergistic) effects. This can allow the in-vitro dose of any individual compound to be greatly lowered without sacrificing effectiveness.

There are a few reports suggesting that some small combinations of two or three natural agents produce synergistic effects *in vivo* without problematic toxicity. For example, Dore et al. (U.S. Patent No. 5,547,947) describe synergistic effects for combinations of two agents: all-*trans*-retinoic acid (ATRA) and a vitamin D_3 metabolite. Intraperitoneal

administration every other day of very large doses of ATRA (300 mg/kg) and 1,25-D_3 (2 Πg/kg) inhibited the growth of human breast cancer cells injected into mice. This was better than the inhibition caused by either agent separately. These doses were the highest that could be used without causing acute side effects. The human equivalents of these doses are approximately 2.2 grams per day of ATRA and 14 micrograms per day of 1,25-D_3 (about 3,7000 I.U. of vitamin D_3). These doses are quite high by human standards, since the average ATRA dose in clinical studies is roughly 300 to 400 milligrams per day and vitamin D_3 can cause toxicity in humans at doses above about 600 I.U. per day. In spite of these high doses, Dore et al. found that the combination of ATRA and 1,23-D_3 did not greatly increase the toxicity of either compound.

Curcumin was found to act synergistically with ATRA and 1,25-D_3 to induce differentiation in human leukemia cells (Liu, Y., et al. Oncology Research (1997) 9:19-29). For example, whereas 10 nM of ATRA induced differentiation in only 10 percent of cells, and 10 ΠM of curcumin induced differentiation in only 16 percent of cells, a combination of the two induced differentiation in 65 percent of cells, indicating that the effects on growth inhibition were additive.

Makishima et al. (Leukemia Res. (1991) 15(8): 701-708) reported that 1,25-D_3 (at 24 nM) and ATRA (at 4 nM) markedly enhanced the differentiation of human leukemia cells induced by genistein (at 19 ΠM).

Katagiri et al. (Cell Immunol. (1992) 140(2): 282-294) reported that genistein (at 37 ΠM) increased 1,25-D_3 induced differentiation of human leukemia cells threefold as compared to 1,25-D_3 alone.

Jing et al. (Anticancer Res. (1993) 13(4): 1049-1054) reported that daidzein (at 40 ΠM) increased differentiation of human leukemia cells induced by both ATRA (at 0.1 ΠM) and 1,25-D3 (at 150 nM) by about 55 percent.

Burns et al. (Cancer Res. (1989) 49(12): 3252-3258) reported that low concentrations (10 ΠM) of docosahexaenoic acid (DHA) markedly increased ATRA-induced differentiation (at 1 ΠM) in human leukemia cells.

Verma et al. (Biochem. Biophys. Res. Commun. (1997) 233:692-696) reported that curcumin (at 10 ΠM) and genistein (at 25 ΠM) showed synergistic *in vitro* inhibition of the growth of human breast cancer cells stimulated by estrogen and/or estrogenic pesticides.

Khafif et al. (Carcinogenesis (1998) 19(3): 419-424) reported that combinations of epigallocatechin gallate (EGCG) and curcumin were synergistic against human oral cancer cells. The combination allowed an eightfold reduction in EGCG concentration (from 19 to 2.1 ΠM) and a twofold reduction in curcumin concentration (from 5.2 to 21 ΠM).

Prasad et al. (1996) reported that a mixture of four antioxidant vitamins reduced the growth of tumorigenic acinar cells.

The synergism studies described above were conducted with no more than two to four natural agents in combination. To date, no studies have been reported wherein five or more natural agents have been used on combination to treat cancer.

SUMMARY OF THE INVENTION

Accordingly, it is a general object of this invention to provide compositions for the treatment of cancer, wherein the compositions comprise five or more natural agents.

It is also a general object of this invention to provide an improved method for treating cancer.

A more specific object of this invention is to provide compositions for the treatment of cancer comprising five or more natural agents, wherein

the cytotoxicity of the individual agents are increased relative to the cytotoxicity of the individual agents when used alone.

Yet another object of this invention is to provide compositions comprising five or more natural agents for the treatment of cancer, wherein the amount of each natural agent in the composition is below the amount which causes significant adverse reactions.

A further object of this invention is to provide compositions comprising five or more natural agents, wherein the compositions inhibit cancer cell proliferation.

Another object of this invention is to provide methods of inhibiting cancer cell proliferation by administering an effective amount of a composition comprising five or more natural agents.

Another object of this invention is to provide a method of treating cancer by administering an effective amount of a composition comprising at least five natural agents.

Additional objects, advantages and novel features of this invention shall be set forth in part in the description that follows, and in part will become apparent to those skilled in the art upon examination of the following specification or may be learned by the practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities, combinations, compositions, and methods particularly pointed out in the appended claims.

To achieve the foregoing and other objects and in accordance with the purposes of the present invention, as embodied and broadly described therein, the present invention provides compositions comprising five or more natural agents combined into one formulation, wherein each of the natural agents has known anticancer activities and wherein the natural agents in the composition interact additively and/or synergistically to enhance their anticancer activities.

To further achieve the foregoing and other objects and in accordance with the purposes of the present invention, as embodied and broadly described therein, the present invention further comprises administering to a mammalian host a therapeutically effective amount of a composition of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and form a part of the specification, illustrate the preferred embodiments of the present invention, and together with the description serve to explain the principles of the invention.

In the Drawings:

Figure 1 is a graphic representation of the effect of a composition of this invention comprising twelve natural agents on inhibition of human breast cancer cells (curve 10), compared to the additive effect of the twelve individual natural agents (curve 12);

Figure 2 is a graphic representation of the effect of a composition of this invention comprising twelve natural agents on inhibition of human prostate cancer cells (curve 14), compared to the additive effect of the twelve individual natural agents (curve 16); Figure 3 is a graphic representation of the effect of a composition of this invention comprising nine natural agents on inhibition of human breast cancer cells (curve 18), compared to the additive effect of the nine individual natural agents (curve 20);

Figure 4 is a graphic representation of the effect of a composition of this invention comprising nine natural agents on inhibition of human prostate cancer cells (curve 22), compared to the additive effect of the nine individual natural agents (curve 24);

Figure 5 is a graphic representation of the effect of a composition of this invention comprising seven natural agents on inhibition of human breast cancer cells (curve 26), compared to the additive effect of the seven individual natural agents (curve 28);

Figure 6 is a graphic representation of the effect of a composition of this invention comprising seven natural agents on inhibition of human prostate cancer cells (curve 30), compared to the additive effect of the seven individual natural agents (curve 32);

Figure 7 is a graphic representation of IC_{50} ratios (IC_{50} additive/IC_{50} observed) verses the number of natural agents in compositions of this invention obtained after testing for inhibition of human breast cancer cells (circles) and human prostate cancer cells (triangles); and

Figure 8 is a graphic representation of the allowed reductions in concentration of natural agents in a composition of this invention versus the number of natural agents in the composition for inhibition of human breast cancer cells (circles) and human prostate cancer cells (triangles).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides methods of inhibiting cancer cell proliferation comprising administering to the cancer cells a therapeutically effective amount of a composition of this invention. The present invention further provides method of treating cancer, comprising administering to a mammalian host a therapeutically effective amount of a composition of this invention. In general, the compositions of this invention comprise five or more natural agents combined into one formulation, wherein each of the natural agents possesses anticancer activity While not wishing to be bound by any theory, it is believed that the natural agents in the compositions interact additively and/or synergistically to enhance the anticancer activities of the other natural agents in the composition. Because of these interactions between the

natural agents of the compositions, it was discovered that the concentration of each natural agent in a composition of this invention could be significantly reduced compared to dose required for effective treatment if each natural agent were administered alone, as discussed below in detail.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

As used herein, the term "additive" refers to an effect of a compositions of this invention wherein the natural agents in the composition interact with each other to produce a higher degree of growth inhibition on cancer cells than can be achieved by a single natural agent at the same dose.

As used herein, the term "synergistic" refers to a coordinated or correlated action of two or more agents or physiologic processes, such that the combined action is greater than the sum of each acting separately. That is, the effects of some or all of the natural agents in compositions of this invention are supra-additive, rather than additive.

As used herein, the term "natural agent" refers generally to a natural compound, that is, a compound that is not synthetically or chemically prepared, having anticancer activity. Thus, "natural agent" refers to a compound obtained from leaves, seeds, bark, fruit, peel, flowers, roots, stems, and/or bulbs of plants, including trees, vegetables, fruits and herbs. In addition, natural agents may by obtained from fish oils. In particular, the natural agents suitable for use in the synergistic

compositions of this invention are preferably saccharides, lipids, amino acids and related thiol-containing compounds, selenium, proteolytic enzymes, phenolic compounds, isoprenoids, saponins, vitamins, and melatonin, as discussed below in detail.

As used herein, the term "cancer" refers to any neoplastic disorder, including such cellular disorders as, for example, carcinomas, sarcomas, and carsinosarcomas and lymphomas. Specific types of cancers include, without limitation, glioma, gliosarcoma, anaplastic astrocytoma, medulloblastoma, lung cancer, small cell lung carcinoma, cervical carcinoma, colon cancer, rectal cancer, chordoma, throat cancer, Kaposi's sarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, colorectal cancer, endometrium cancer, ovarian cancer, breast cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, hepatic carcinoma, bile duct carcinoma, choriocarcinoma, seminoma, testicular tumor, Wilms' tumor, Ewing's tumor, bladder carcinoma, angiosarcoma, endotheliosarcoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland sarcoma, papilary sarcoma, papilary adenosarcoma, cystadenosarcoma, bronchogenic carcinoma, medullary carcinoma, mastocytoma, mesothelioma, synovioma, melanoma, leiomyosarcoma, rhabdomyosarcoma, neuroblastoma, retinoblastoma, oligodentroglioma, acoustic neuroma, hemangioblastoma, meningioma, pinealoma, ependymoma, craniopharyngioma, epithelial carcinoma, embryonal carcinoma, squamous cell carcinoma, base cell carcinoma, fibrosarcoma, myxoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, leukemia, and the metastatic lesions secondary to these primary tumors. In general, any neoplastic lesion, including granulomas, may be treated according to the present invention.

As used herein, an "effective amount" or "effective dose" is an amount of the synergistic composition to be administered to the host that kills cancer cells or inhibits the proliferation thereof, or which reduces

tumor size. Suitable doses of a synergistic composition can be determined by various methods, including generating an empirical dose-response curve, and other methods used in the pharmaceutical sciences.

A basis of the present invention is the finding that combinations of five or more natural agents in the compositions of this invention work additively and/or synergistically to enhance the anticancer activities of the other agents in the composition. Further, it was discovered that increasing the number of natural agents in a composition of this invention dramatically decreased the concentration of each natural agent required in the composition to provide a effective dose of the composition, compared to when the individual natural agent was used. For example, it was discovered that compositions comprising seven, nine, and twelve different natural agents could be prepared having increasing abilities to inhibit cancer cell proliferation. This offers the advantage of allowing much lower doses, far below the toxic doses of the natural agents, to be used as compared to the dose of a single natural agent that would be required in order to achieve the same degree of inhibition of cancer cell proliferation. Until this invention, it was not known that five or more natural agents, having different anticancer activities, could be combined into one formulation that would possess the ability to inhibit cancer cell proliferation. In fact, until this invention it was thought that, in compositions comprising more than four natural agents, the natural agents might cancel each other's anticancer activities or worse, enhance cancer cell proliferation.

As stated above, the compositions of this invention comprise five or more natural agents combined into one formulation. In particular, the natural agents suitable for use in the compositions of this invention are preferably saccharides, lipids, amino acids and related thiol-containing compounds, selenium, proteolytic enzymes, phenolic compounds, isoprenoids, saponins, vitamins, and melatonin.

Non-limiting examples of saccharides or sources of saccharides suitable for use in this invention include monosaccharides, such as vitamin C; disaccharides; oligosaccharides; and polysaccharides, such as polysaccharide K (PSK), and polysaccharide peptide (PSP), and polysaccharides derived from plants such *Astragelus membranaceus*, *Elutherococcus senticocus*, *Ganoderma lucidum*, and Shitake (*Lentinus edodes*).

Non-limiting examples of lipids suitable for use in this invention include omega-3 fatty acids such as eicosapenaenoic acid (EPA) and docosahexaenoic acid (DHA).

Non-limiting examples of amino acids suitable for use in this invention include glutathione and glutamine.

Non-limiting examples of thiol-containing compounds suitable for use in this invention include thiol compounds derived from garlic, such as allicin, diallyl disulfide (DADS), diallyl trisulfide (DATS), S-allylcysteine (SAC), and S-allylmercaptocysteine (SAMC).

Non-limiting examples of proteolytic enzymes suitable for use in this invention include bromelain, trypsin, and chymotrypsin.

Non-limiting examples of phenolic compounds or sources of phenolic compounds suitable for use in this invention include flavonoids including flavanones such as tangeretin and naringin; flavones such as luteolin and apigenin; isoflavones such as genistein and diadzein; flavonols, including quercetin, catechins (e.g., epigallocatechin gallate (EGCG), epicatechin, epicatechin gallate, and epigallocatechinquercetin), and kaemferol; anthocyanidins; proanthocyanidins; caffeic acid esters including caffeic acid phenethyl ester (CAPE), caffeic acid benzyl ester, methyl caffeate, phenethyl dimethylcaffeate, and other related caffeic acid esters; curcumin; lignans such as ursolic acid, asiatic acid, arctigenin and flax

seed (*Linum usitatissimum*); silbenes such as resveratrol; and quinones such as emodin and hypercin.

Non-limiting examples of isoprenoids or sources of isoprenoids suitable for use in this invention include terpenes, including monoterpenes such as limonene, perillyl alcohol, and geraiol; triterpenes such as boswellic acid and *Centella asiatica*; and sesquiterpenes.

Non-limiting examples of saponins or sources of saponins suitable for use in this invention include horse chestnut, butcher's broom (*Ruscus aculeatus*), and ginseng.

Non-limiting examples of vitamins suitable for use in this invention include vitamin A, vitamin D_3, and vitamin E.

It is preferred that the natural agents used in the synergistic compositions of the present parthenolide.invention meet the following criteria:

- θ the natural agent is not currently approved as a prescription drug by the United States Food and Drug Administration;
- θ the natural agent (or their plant sources) has a history of safe human use as food or in herbal medicine traditions;
- θ the natural agent is active at a concentration that is achievable in humans;
- θ the natural agent is not toxic to the patient at the required dose;
- θ the natural agent is suitable for long-term therapy; and
- θ the natural agent may be administered orally.

The natural agents suitable for use in the compositions of this invention may be associated with three primary categories based on their mechanisms of action. These categories include 1) direct inhibitors, that is, compounds that have a cytotoxic or cytostatic effect and can directly

inhibit cancer cells, or compounds that directly inhibit the ability of growth factors to promote cancer growth; 2) compounds that act by indirect means, including compounds that inhibit angiogenesis, invasion, and metastasis; and 3) compounds that stimulate the immune system. Non-limiting examples of natural agents that fall into these three categories are listed in Table 1. However, many of the natural agents suitable for use in the compositions of this invention fall into more than one category. For example, many direct-acting compounds also have indirect actions, such as anti-inflammatory actions. Accordingly, in one embodiment of this invention, a composition of this invention inhibits cancer by affecting multiple mechanisms of this invention, that is, the composition comprises natural agents selected from all three categories shown in Table 1. While not wishing to be bound by theory, it is believed that by affecting multiple mechanisms, the compositions of this invention may inhibit a wider range of cancers under a greater variety of circumstances.

TABLE 1

THERAPEUTIC CATEGORY	NATURAL AGENTS
Immune stimulants	*Astragalus*, bromelain, *Eleutherococcus*, *Ganoderma*, ginseng, melatonin, PSP, PSK, selenium, shitake
Indirect-acting compounds	anthocyanidins, butcher's broom, *Centella*, EPA, DHA, glutamine, horse chestnut, proanthocyanidins, vitamin C
Direct-acting compounds	Vitamin A, apigenin, arctigenin, boswellic acid, CAPE, curcumin, EGCG, emodin, flaxseed, genistein, daidzein, garlic hypercin, luteolin, terpenes, parthenolide, resveratrol, vitamin D, vitamin E

The natural agents used in the compositions of this invention may be provided in the form of pure substances, or as concentrated plant extracts containing the natural agents in concentrations between about 20 to 80 percent. In one embodiment, the compositions of this invention comprise approximately equivalent concentrations of each natural agent. In another embodiment, the compositions of this invention comprise different amounts of each natural agent. The amount of natural agent contained in a composition of this invention will depend in part on the type of cancer being treated, the desired results of the treatment, the stage of the cancer and the health of the patient. In specific embodiments described herein, three compositions comprising various combinations of natural agents were prepared, having the formulations shown in Table 2. Combination C3 contained twelve natural agents, contained C4 comprised nine natural agents, and combination C2 contained seven natural agents.

Compositions C3, C4, and C2 shown in Table 2 were tested for their ability to inhibit cancer cell proliferation in various cell lines, and the results were compared with the additive inhibitions of the individual agents. The tumor models chosen for the present studies are the human breast cancer cell line MCF7 and the human prostate cancer cell line PC3.

Composition C3, comprising equal amounts (after adjusting for purity) of the twelve natural agents in the amounts shown in Table 2, was tested for its ability to inhibit the proliferation of MCF7 human breast cancer cells *in vitro*, and the results were compared to the additive inhibitions of the individual agents contained in composition C3. The results are illustrated in Figure 1. The 50 percent inhibition (IC_{50}) line is shown by the horizontal dotted line in Figure 1. Curve 10 in Figure 1 represents the observed ability of composition C3, comprised of a combination of 12 natural agents, to inhibit growth of human breast cancer cells. Curve 12 in Figure 1 represents the additive inhibition of the 12 individual natural agents. Curve 12 is mathematically computed based

on combining the individual cell inhibition values observed for, apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, luteolin, CAPE, resveratrol, EGCG, and parthenolide. In this composition, ATRA (all-*trans*-retinoic acid), a metabolite formed soon after administration of vitamin A, was used instead of vitamin A to mimic *in vivo* conditions of vitamin A. In a final therapeutic composition, ATRA would be replaced by vitamin A in this formulation.

Since all natural agents in the composition C3 were at equal concentrations (after adjusting for purity), curve 12 is also representative of an "average" natural agent of the composition C3. Curve 12 in Figure 1 shows that the average individual natural agent only marginally inhibits cell growth at low concentrations. In fact, cell proliferation in the presence of an individual natural agent remained near 90 percent until the concentration of the compound reached about 1 Πg/ml. At concentrations of an individual natural agent above 1 Πg/ml, cell proliferation begins to decline rapidly. This is important because it implies that administration of a low dose of an individual natural agent, which would produce a low plasma concentration, would have little effect on cancer cell proliferation. In contrast, curve 10, which is the observed inhibition of cell proliferation by composition C3, shows that cell proliferation in the presence of 1 Πg/ml of composition C3 is dramatically lower (approximately 65 percent) when compared to the same concentration of an individual natural agent.

Another important discovery illustrated by Figure 1 is that the observed IC_{50} for composition C3 (curve 10) is 4.0 Πg/ml (13 ΠM), whereas the additive IC_{50} for the additive curve (curve 12) is significantly higher at 18 Πg/ml (58 ΠM). The IC_{50} ratio (IC_{50} additive/IC_{50} observed) is therefore 18/4.0 or 4.5. This 4.5-fold difference between the observed IC_{50} (curve 10) and the additive IC_{50} curve 12 is quite remarkable, and shows that supra-additive (synergistic) effects may be occurring among the twelve agents in composition C3. That is, the IC_{50} ratio would have been 1 (i.e., a

horizontal line) if the effects of the composition C3 were purely additive. Prior to this invention, such a large combination of agents had never been tested for its ability to inhibit cancer cell proliferation. Further, it was not known prior to this invention that such a large combination of agents would provide a significantly lower IC_{50} when tested against cancer cells, or rather if the natural agents would cancel out each other's anticancer activities or even enhance cancer cell proliferation.

Composition C3 comprising twelve natural agents was also tested for its ability to inhibit proliferation of PC3 human prostate cancer cells and was compared to the additive cell inhibition of the individual natural agents. The results are illustrated in Figure 2. Again, the results show that composition C3 (curve 14) exhibits synergistic (supra-additive) effect in its ability to inhibit human prostate cancer cell proliferation compared to the additive inhibitions of the twelve individual natural agents (curve 16). The observed IC_{50} for composition C3 (curve 14) is 5.7 Πg/ml, whereas the additive IC_{50} for the additive curve (curve 16) is higher at 13.1 Πg/ml. The IC_{50} ratio (IC_{50} additive/IC_{50} observed) is therefore 13.1/5.7 or 2.3.

Figure 3 illustrates the ability of composition C4, comprising nine natural agents (Table 2) to inhibit MCF7 human breast cancer cell proliferation compared to the additive inhibitions of the individual agents that make up composition C4. Curve 18 in Figure 3 is the observed ability of composition C4, comprised of a combination of nine natural agents, to inhibit growth of human breast cancer cells. Curve 20 in Figure 3 represents the average additive inhibition of the individual agents (i.e., apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, and CAPE) that make up the composition. The observed IC_{50} for composition C4 (curve 18) is 6.4 Πg/ml, whereas the additive IC_{50} for the additive curve (curve 20) is much higher at 21.7 Πg/ml. The IC_{50} ratio (IC_{50} additive/IC_{50} observed) is therefore 21.7/6.4 or 3.4, demonstrating that composition C4

exhibits synergistic activity against human breast cancer cell proliferation compared to the individual components of composition C4.

Table 2

Natural agent	Composition C3 concentration of natural agent (μg/mL)	Composition C4 concentration of natural agent (μg/mL)	Composition C2 concentration of natural agent (μg/mL)
Vitamin A	1.9	2.9	-
Apigenin	5.6	8.5	7.09
Arctigenin	3.8	5.8	4.82
Boswellic acid	3.0	4.5	3.73
Curcumin	2.0	3.0	2.50
Emodin	2.5	3.8	3.16
Genistein H	-	-	4.89
Genistein S	1.9	2.9	-
Luteolin	1.9	-	-
CAPE (from Propolis)	8.7	-	11.02
Resveratrol	1.9	-	-
EGCG	1.9	2.9	-
Parthenolide	1.9	2.9	-
Total number of agents	12	9	7

Figure 4 illustrates the ability of composition C4 (Table 2) to inhibit PC3 human prostate cancer cell proliferation compared to the additive inhibitions of the individual agents apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, and CAPE. Curve 22 (dashed curve) in Figure 4 is the observed ability of composition C4, comprised of a combination of nine natural agents, to inhibit growth of human breast cancer cells. Curve 24 (solid curve) in Figure 4 represents the average additive inhibition of the individual agents. The observed IC_{50} for composition C4 (curve 22) is 7.2 Πg/ml, whereas the additive IC_{50} for the additive curve (curve 24) is much higher at 16.7 Πg/ml. The IC_{50} ratio (IC_{50} additive/IC_{50} observed) is therefore 16.7/7.2 or 2.3, demonstrating that composition C4 exhibits synergistic activity against human prostate

cancer cell proliferation compared to the individual components of composition C4.

Figures 5 and 6 illustrate the ability of composition C2, comprising 7 natural agents (Table 2) to inhibit MCF7 human breast cancer cells and PC3 human prostate cancer cell proliferation, respectively, compared to the additive inhibitions of the individual agents apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, and CAPE. For composition C2, the IC_{50} ratio (IC_{50} additive/IC_{50} observed) from the human breast cancer cell inhibition study was 21.8/10.5 or 2.1, and the IC_{50} ratio for the human prostate cancer cell inhibition study was 17.8/7.6 or 2.3, demonstrating that compositions comprising seven natural agents exhibit synergistic rather than additive effects in their ability to inhibit cancer cell proliferation.

Figure 7 shows a graph of IC_{50} ratios (IC_{50} additive/IC_{50} observed) plotted for various compositions of this invention after testing both the synergistic compositions and the individual natural agents for their ability to inhibit both human breast cancer cells (circles) and human prostate cancer cell lines (triangles) *in vitro*. As can be seen in Figure 7, an increase in the number of natural agents in the composition produces an increase in the IC_{50} ratio for human breast cancer cells (circles). That is, the IC_{50} ratios are higher than would be expected if the agents in the composition only interacted additively, indicating that the agents in the compositions of this invention are interacting in a synergistic manner. The IC_{50} ratio for human prostate cancer cells (triangles) increases from 1.0 to 2.3 as the number of agents in the composition increases from one to seven, and then remains as 2.3 as the number of natural agents in the composition increases to greater than seven agents.

The data illustrated in Figure 7 suggests that the ability of different compositions of this invention depend in part on the particular natural agents used in the composition, and in part on the number of natural

agents in the composition. Moreover, Figure 7 shows that synergistic effects are not limited to a single cell line. In addition, the results in both cell lines were more than additive. If purely additive effects had occurred, the curves for both cell lines in Figure 7 would have been horizontal. In other words, if the anticancer activities of the natural agents in the formulation were merely additive, the IC_{50} ratio would have been 1.0.

The data shown in Figure 7 also allows for an estimation of the degree of reduction in concentration possible in the compositions of this invention, by multiplying the number of agents in a combination by the ratio of the IC_{50}. For example, the results obtained for the inhibition of human breast cancer cell proliferation (circles) show that a combination of nine natural agents produces an IC_{50} ratio of 3.4. That is, a composition comprising nine natural agents, combined in the formulation in equal concentrations (i.e., each natural agent comprises one-ninth the total concentration) is effective at a concentration that is 3.4-fold lower than that of a single compound. Therefore, the total *in vitro* reduction in concentration allowed for each compound in the 9-compound composition is 9ט3.4 = 31. That is, a 31-fold reduction in the concentration of each natural agent is possible when the natural agents are combined in a composition of this invention, as compared to the amount of a single natural agent that would be required to achieve the same percent inhibition of cancer cell growth.

The calculated allowed reductions for various compositions for both human breast cancer and human prostate cancer cell lines are shown in Figure 8. Figure 8 shows that, for example, the concentration of each natural agent in a synergistic composition of this invention comprising eight natural agents can be reduced by at least approximately 18-fold. These results are significant, since a 20-fold reduction in the required concentration of a natural agent would dramatically reduce the amount of natural agents that would need to be administered to a patient in order to

provide effective therapy. In other words, the effective dose of a natural agent in a composition of this invention is significantly lower that the effective dose of a single natural agent. Consequently, lower and non-toxic concentrations of natural agents may be used in the compositions of this invention, while providing significant inhibition of cell proliferation.

Overall, the results discussed above demonstrate that compositions of this invention comprising five or more natural agents afford appreciable inhibition of cancer cell proliferation as compared to the additive inhibitions of the individual natural agents. That is, the sum of the effects of the compositions of this invention is greater than that produced by any one agent used singularly. Consequently, each natural agent in the composition can be used at a more moderate dose compared to the dose required if the agents were used singularly, preferably at a dose low enough to preclude adverse side affects. Thus, this invention show that it is possible to create highly effective combinations using a large number natural agents, and that the additive and/or synergistic effect of the natural agents in the compositions of this invention generally increases with an increasing number of natural agents in the composition.

The compositions of this invention are useful for inhibiting cancer cell proliferation. The present invention further provides methods of treating cancer comprising administering a composition of this invention to a host in need of cancer therapy. The doses, routes of administration, and carriers and/or adjuvants used may vary based on the type of cancer being treated and in view of known procedures for treatment of such cancers.

Preferably, the compositions of this invention are administered orally; however, parentaral administration can be used. While it is possible to administer the compositions of this invention alone, the compositions may also be administered as part of a pharmaceutical formulation. For oral administration, the compositions of this invention

can be used in the form of tablets, capsules, granules, powders, lozenges, syrups, elixirs, solutions, suspensions, and the like, in accordance with standard pharmaceutical practice.

For parenteral administration, which includes intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the natural agents are usually prepared, and the pH of the solutions are suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic.

Carriers useful in formulating the preparations are commonly used pharmaceutically acceptable non-toxic carriers such as gelatin, lactose sodium citrate, salts of phosphoric acid, starch, magnesium stearate, sodium lauryl sulfate, talc, polyethylene glycol, etc. The carrier may be used with other additives such as diluents, binders, buffer agents, preservatives, sweetening agents, flavoring agents, glazes, disintegrators, coating agents emulsifying agents, suspending agents, etc.

The dosage regiment may be regulated according to the potency of the individual natural agents utilized in the compositions of this invention, the mode of administration, and the needs of the host depending on factors such as the degree and severity of the disease state and age and general condition of the host being treated. Determination of dose amount for a particular administration is within the skill of the art.

EXAMPLES

The source and purities of the natural agents used in formulations C3, C2 and C4 in Table 2 are as follows: ATRA (100%; Sigma), apigenin (34.2%; Hauser, Inc.), arctigenin (50.3%; Hauser, Inc.), boswellic acid (65%), curcumin (97%), emodin (76.6%; Hauser, Inc.), genistein H (49.6%; Hauser), genistein S (100%; Sigma), luteolin (100%; Sigma), CAPE (from propolis, 22%; Hauser, Inc.), resveratrol (100%; Sigma), EGCG (100%;

Sigma), partenolide (100%; Sigma).

Example 1. Solubility and initial cytotoxicity determination:

The goal of this experiment was to determine a solubilizing agent compatible with *in vitro* cell growth. The solubilities of each of the individual natural agents to be used in the compositions of this invention were examined. The maximum solubility of each material was determined using dimethyl sulfoxide (DMSO) or saline as the solubilizing agent. If the natural agent was soluble in DMSO as opposed to saline, further dilutions in saline or tissue culture medium were performed. In all cases, the DMSO concentration in the final experiment never exceeded 0.2%, a concentration which did not by itself affect either cell growth or viability.

Example 2: Inhibition assays of the compositions comprising natural agents:

Using appropriate dilutions of stock concentrations of each natural agent, the IC50 and IC10 concentrations were determined. These represent those concentrations of agent that resulted in 50% or 10% inhibition of cell growth, respectively, over a 72 hour period when cells were exposed continuously to compound. Inhibition of cell growth was determined by comparison to control cell populations which were not treated with the natural agents. At the end of the 72 hour incubation period, relative cell growth was determined using the "MTT" methylenetetrazolium dye assay as described by Mossmann (J. Immunol. Methods, 1983, 65: 55). Cell viability was measured as a function of the ability of cells to form a blue formazan product, the optical density of which was determined by a Dynatech Microplate reader (570 nm; reference set at 630 nm).

The efficacies of compositions of this invention were determined through simultaneous incubation of the IC10 concentrations of each compound with a given cell line. In some instances the initial concentration of a specific compound had to be lowered to avoid excessive

inhibition of cell growth. Experiments were repeated a minimum of three times.

The foregoing description is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and process shown as described above. Accordingly, all suitable modifications and equivalents may be resorted to falling within the scope of the invention as defined by the claims that follow.

The words "comprise," "comprising", "include," "including," and "includes" when used in this specification and in the following claims are intended to specify the presence of stated features, integers, components, or steps, but they do not preclude the presence or addition of one or more other features, integers, components, steps, or groups thereof.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A composition for treating cancer, said composition comprising a mixture of at least five natural agents.

2. The composition of claim 1, wherein said composition comprises seven natural agents.

3. The composition of claim 1, wherein said composition comprises nine natural agents.

4 The composition of claim 1, wherein said composition comprises twelve natural agents.

5. The composition of claim 1, wherein said natural agents are selected from the group consisting of saccharides, lipids, amino acids related thiol-containing compounds, selenium, proteolytic enzymes, phenolic compounds, isoprenoids, saponins, vitamins, and melatonin.

6. The composition of claim 5, wherein said saccharides are selected from the group consisting of monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

7. The composition of claim 6, wherein said polysaccharides are selected from the group consisting of *Astragelus membranaceus, Elutherococcus senticocus, Ganoderma lucidum*, shitake, polysaccharide K, and polysaccharide peptide.

8. The composition of claim 5, wherein said lipids are eicosapentaenoic acid or docosahexaenoic acid.

9. The composition of claim 5, wherein said amino acids are glutathione or glutamine.

10. The composition of claim 5, wherein said thiol-containing compounds are allicin, diallyl disulfide, diallyl trisulfice, S-allylcysteine, and S-allylmercaptocysteine.

11. The composition of claim 5, wherein said proteolytic enzymes are bromelain, trypsin, and chymotrypsin.

12. The composition of claim 5, wherein said phenolic compounds are selected from the group consisting of flavanones, flavones, isoflavones, flavonols, anthocyanidins, proanthocyanidins, caffeic acid esters, curcumin, lignans, stilbenes, and quinones.

13. The composition of claim 12, wherein said flavanones are tangeretin or naringin.

14. The composition of claim 12, wherein said isoflavones are genistein or diadzein.

15. The composition of claim 12, wherein said flavanols are selected from the group consisting of quercetin, epigallogallate, epicatechin, epicatechin gallate, epigallocatechinquercetin, and kaemferol.

16. The composition of claim 12, wherein said caffeic acid esters are selected from the group consisting of caffeic acid phenethyl ester, caffeic acid benzyl ester, methyl caffeate, and phenethyl dimethylcaffeate.

17. The composition of claim 12, wherein said lignans are selected from the group consisting of acrigenin, flax seed, silybin, and schizandrin.

18. The composition of claim 5, wherein said isoprenoids are selected from the group consisting of monoterpenes, triterpenes, and sesquiterpenes.

19. The composition of claim 18, wherein said triterpenes are selected from the group consisting of boswellic acid, ursolic acid, asiatic acid, and *Centella asiatica*.

20. The composition of claim 18, wherein said sesquiterpene is parthenolide.

21. The composition of claim 5, wherein said vitamins are selected from the group consisting of vitamin A, vitamin D_3, and vitamin E.

22. The composition of claim 4, wherein said natural agents are vitamin A, apigenin, arctigenin, boswellic acid curcumin, emodin, genistein, luteolin, caffeic acid phenethyl ester, resveratrol, epigallocatechin gallate, and parthenolide.

23. The composition of claim 3, wherein said natural agents are vitamin A, apigenin, arctigenin, boswellic acid curcumin, emodin, genistein, epigallocatechin gallate, and parthenolide.

24. The composition of claim 2, wherein said natural agents are apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, caffeic acid phenethyl ester, and parthenolide.

25. The composition of claim 1, wherein said method of treating cancer comprises inhibiting cancer cell proliferation.

26. A method for inhibiting cancer cell proliferation, comprising contacting said cells with an effective amount of a composition comprising at least five natural agents.

27. The method of claim 26, wherein said composition comprises seven natural agents.

28. The method of claim 26, wherein said composition comprises nine natural agents.

29 The method of claim 26, wherein said composition comprises twelve natural agents.

30. The method of claim 26, wherein said natural agents are selected from the group consisting of saccharides, lipids, amino acids related thiol-containing compounds, selenium, proteolytic enzymes, phenolic compounds, isoprenoids, saponins, vitamins, and melatonin.

31. The method of claim 30, wherein said saccharides are selected from the group consisting of monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

32. The method of claim 31, wherein said polysaccharides are selected from the group consisting of *Astragelus membranaceus, Elutherococcus senticocus, Ganoderma lucidum*, shitake, polysaccharide K, and polysaccharide peptide.

33. The method of claim 30, wherein said lipids are eicosapentaenoic acid or docosahexaenoic acid.

34. The method of claim 30, wherein said amino acids are glutathione or glutamine.

35. The method of claim 30, wherein said thiol-containing compounds are allicin, diallyl disulfide, diallyl trisulfice, S-allylcysteine, and S-allylmercaptocysteine.

36. The method of claim 30, wherein said proteolytic enzymes are bromelain, trypsin, and chymotrypsin.

37. The method of claim 30, wherein said phenolic compounds are selected from the group consisting of flavanones, flavones, isoflavones, flavonols, anthocyanidins, proanthocyanidins, caffeic acid esters, curcumin, lignans, stilbenes, and quinones.

38. The method of claim 37, wherein said flavanones are tangeretin or naringin.

39. The method of claim 37, wherein said isoflavones are genistein or diadzein.

40. The method of claim 37, wherein said flavonols are selected from the group consisting of quercetin, epigallogallate, epicatechin, epicatechin gallate, epigallocatechinquercetin, and kaemferol.

41. The method of claim 37, wherein said caffeic acid esters are selected from the group consisting of caffeic acid phenethyl ester, caffeic acid benzyl ester, methyl caffeate, and phenethyl dimethylcaffeate.

42. The method of claim 37, wherein said lignans are selected from the group consisting of acrigenin, flax seed, silybin, and schizandrin.

43. The method of claim 30, wherein said isoprenoids are selected from the group consisting of monoterpenes, triterpenes, and sesquiterpenes.

44. The method of claim 43, wherein said triterpenes are selected from the group consisting of boswellic acid, ursolic acid, asiatic acid, and *Centella asiatica*.

45. The method of claim 43, wherein said sesquiterpene is parthenolide.

46. The method of claim 30, wherein said vitamins are selected from the group consisting of vitamin A, vitamin D_3, and vitamin E.

47. The method of claim 29, wherein said natural agents are vitamin A, apigenin, arctigenin, boswellic acid curcumin, emodin, genistein, luteolin, caffeic acid phenethyl ester, resveratrol, epigallocatechin gallate, and parthenolide.

48. The method of claim 28, wherein said natural agents are vitamin A, apigenin, arctigenin, boswellic acid curcumin, emodin, genistein, epigallocatechin gallate, and parthenolide.

49. The method of claim 27, wherein said natural agents are apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, caffeic acid phenethyl ester, and parthenolide.

50. A method of treating a host by inducing inhibition of cancer cell proliferation in a tumor, said method comprising administering to said host an effective amount of a composition comprising at least five natural agents.

51. The method of claim 50, wherein said tumor is a human breast tumor.

52. The method of claim 50, wherein said tumor is a human prostate tumor.

53. The method of claim 50, wherein said composition comprises seven natural agents.

54. The method of claim 50, wherein said composition comprises nine natural agents.

55. The method of claim 50, wherein said composition comprises twelve natural agents.

56. The method of claim 50, wherein said natural agents are selected from the group consisting of saccharides, lipids, amino acids related thiol-containing compounds, selenium, proteolytic enzymes, phenolic compounds, isoprenoids, saponins, vitamins, and melatonin.

57. The method of claim 56, wherein said saccharides are selected from the group consisting of monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

58. The method of claim 57, wherein said polysaccharides are selected from the group consisting of *Astragelus membranaceus, Elutherococcus senticocus, Ganoderma lucidum*, shitake, polysaccharide K, and polysaccharide peptide.

59. The method of claim 56, wherein said lipids are eicosapentaenoic acid or docosahexaenoic acid.

60. The method of claim 56, wherein said amino acids are glutathione or glutamine.

61. The method of claim 56, wherein said thiol-containing compounds are allicin, diallyl disulfide, diallyl trisulfice, S-allylcysteine, and S-allylmercaptocysteine.

62. The method of claim 56, wherein said proteolytic enzymes are bromelain, trypsin, and chymotrypsin.

63. The method of claim 56, wherein said phenolic compounds are selected from the group consisting of flavanones, flavones, isoflavones, flavonols, anthocyanidins, proanthocyanidins, caffeic acid esters, curcumin, lignans, stilbenes, and quinones.

64. The method of claim 63, wherein said flavanones are tangeretin or naringin.

65. The method of claim 63, wherein said isoflavones are genistein or diadzein.

66. The method of claim 63, wherein said flavonols are selected from the group consisting of quercetin, epigallogallate, epicatechin, epicatechin gallate, epigallocatechinquercetin, and kaemferol.

67. The method of claim 63, wherein said caffeic acid esters are selected from the group consisting of caffeic acid phenethyl ester, caffeic acid benzyl ester, methyl caffeate, and phenethyl dimethylcaffeate.

68. The method of claim 63, wherein said lignans are selected from the group consisting of acrigenin, flax seed, silybin, and schizandrin.

69. The method of claim 56, wherein said isoprenoids are selected from the group consisting of monoterpenes, triterpenes, and sesquiterpenes.

70. The method of claim 69, wherein said triterpenes are selected from the group consisting of boswellic acid, ursolic acid, asiatic acid, and *Centella asiatica*.

71. The method of claim 69, wherein said sesquiterpene is parthenolide.

72. The method of claim 56, wherein said vitamins are selected from the group consisting of vitamin A, vitamin D_3, and vitamin E.

73. The method of claim 55, wherein said natural agents are vitamin A, apigenin, arctigenin, boswellic acid curcumin, emodin,

genistein, luteolin, caffeic acid phenethyl ester, resveratrol, epigallocatechin gallate, and parthenolide.

74. The method of claim 54, wherein said natural agents are vitamin A, apigenin, arctigenin, boswellic acid curcumin, emodin, genistein, epigallocatechin gallate, and parthenolide.

75. The method of claim 53, wherein said natural agents are apigenin, arctigenin, boswellic acid, curcumin, emodin, genistein, caffeic acid phenethyl ester, and parthenolide.

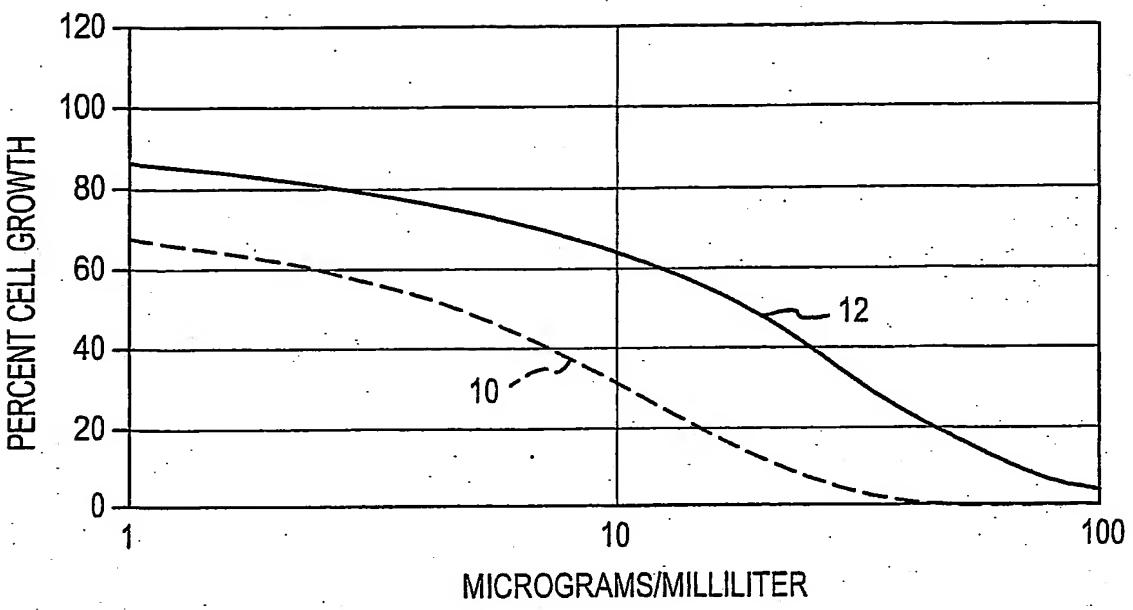

FIG.1

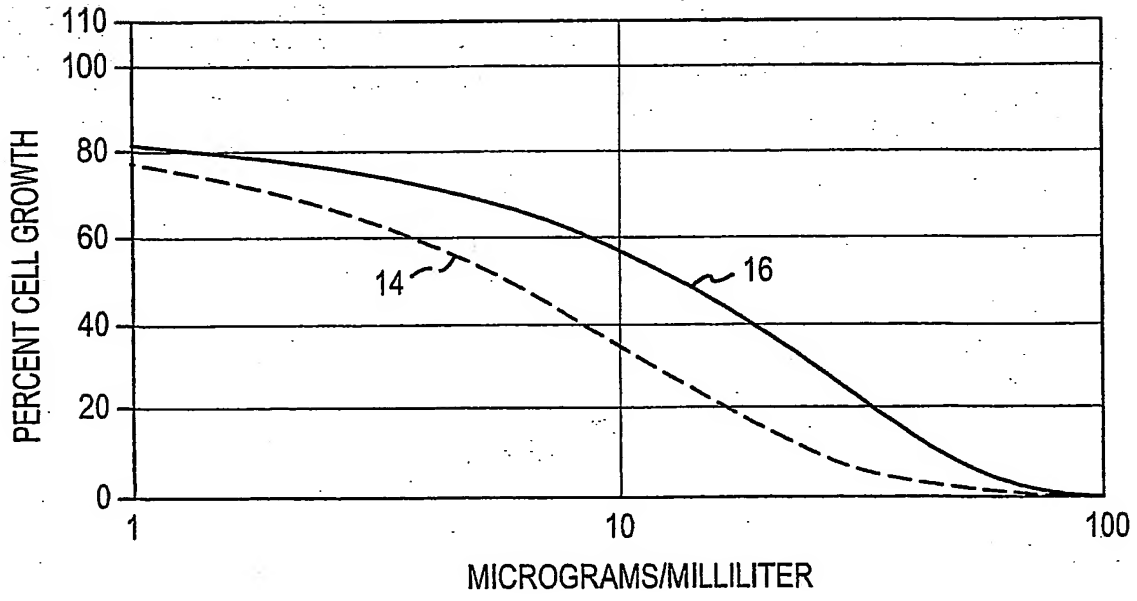

FIG.2

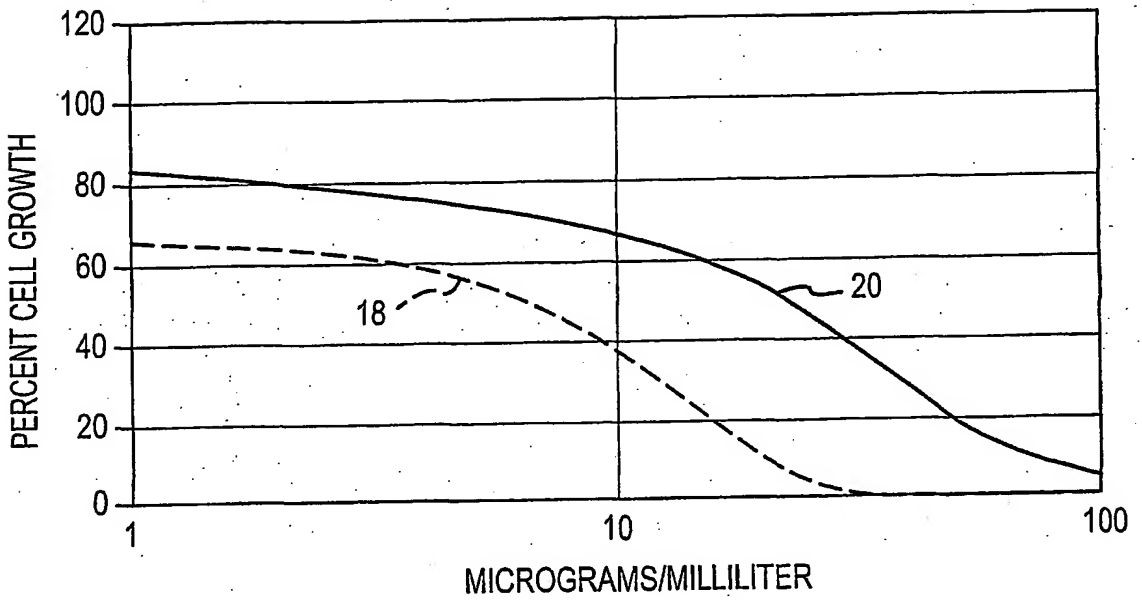

FIG.3

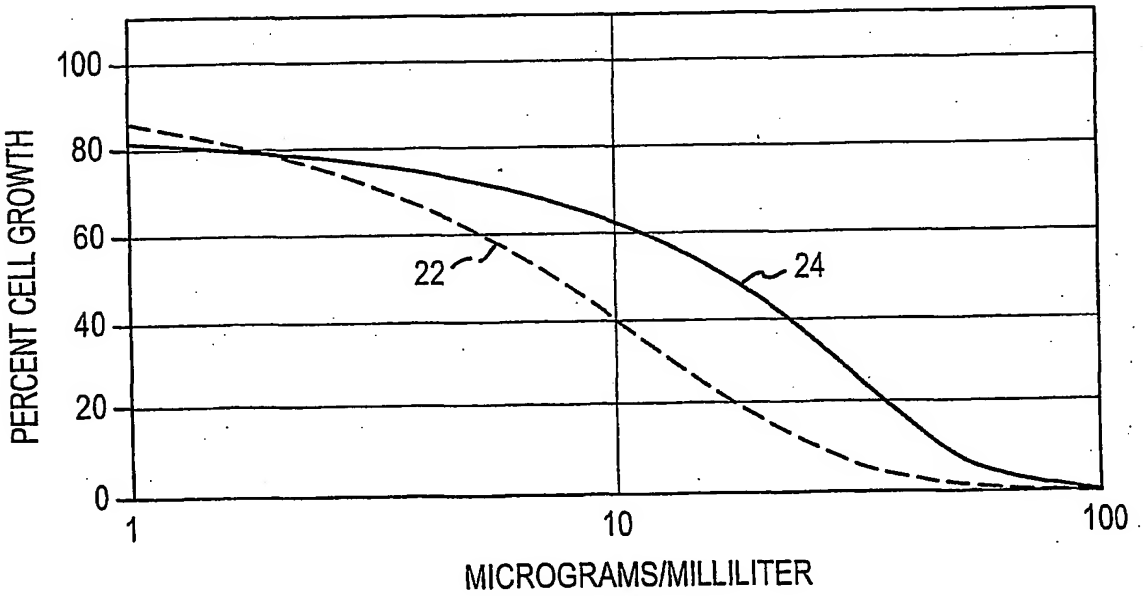

FIG.4

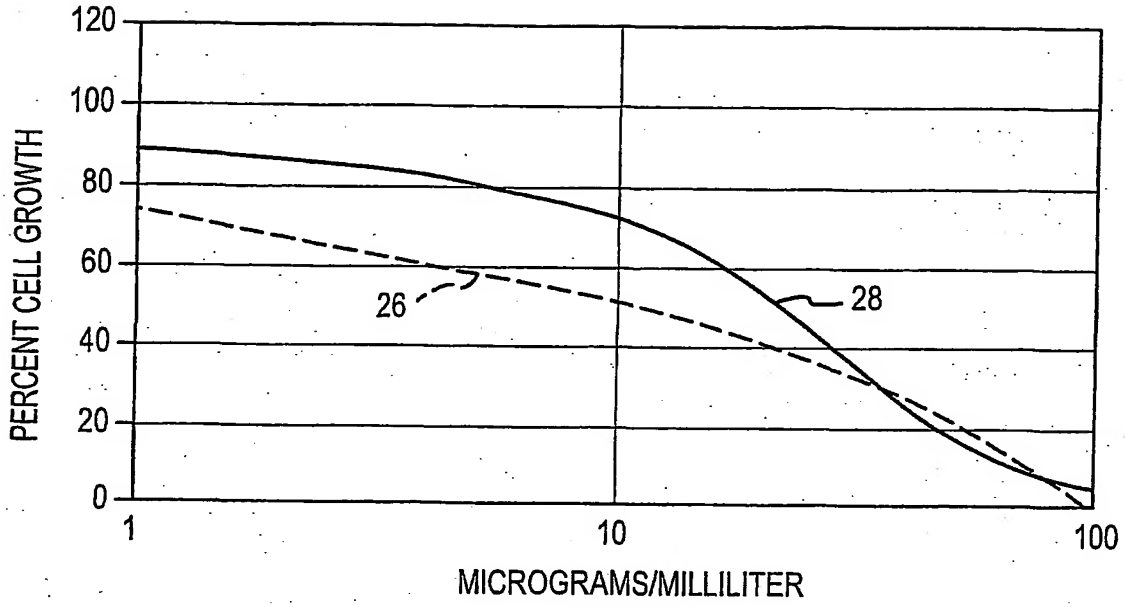

FIG.5

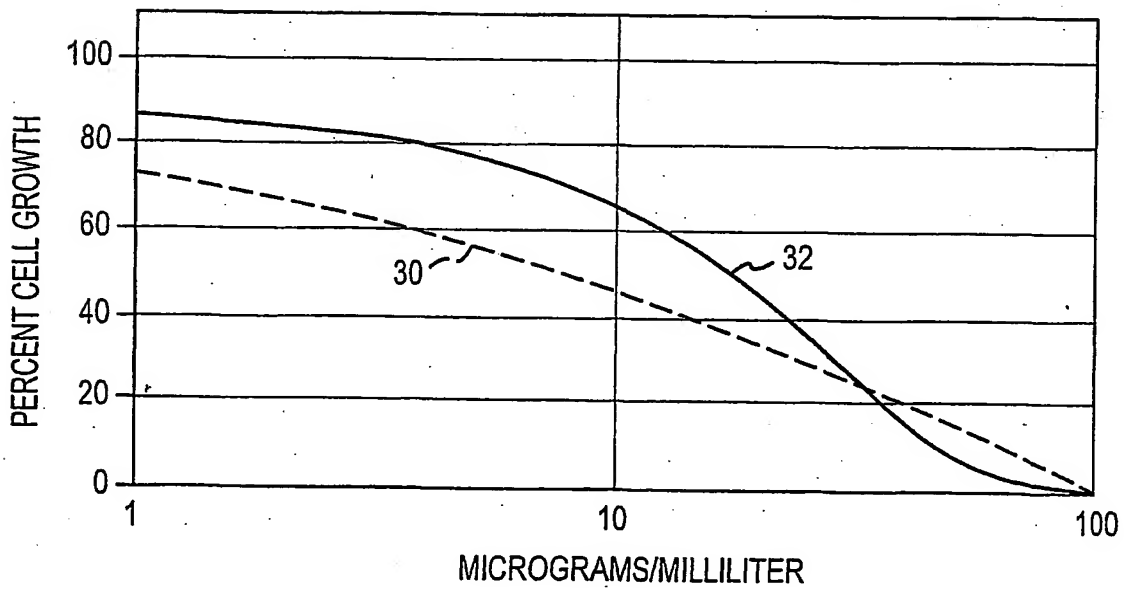

FIG.6

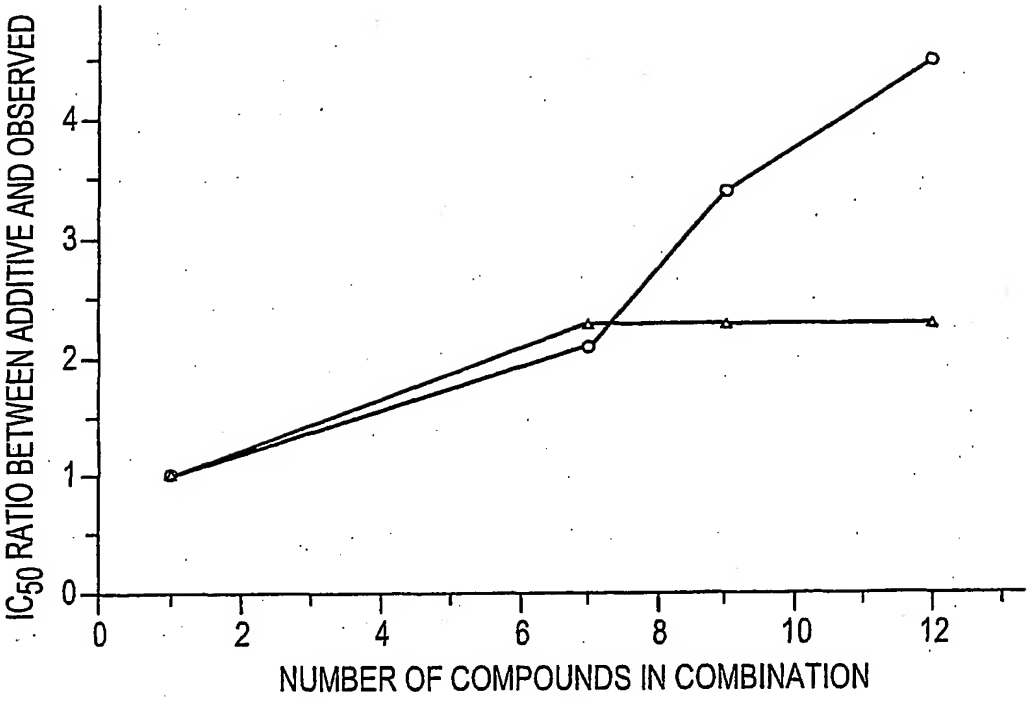

FIG.7

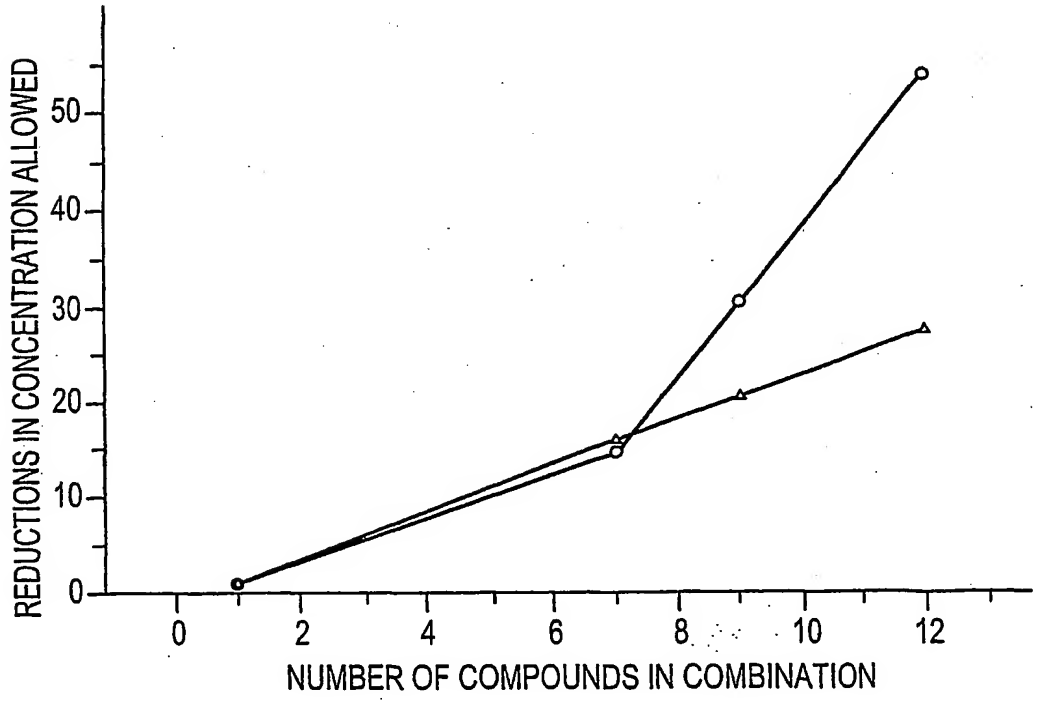

FIG.8

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(54) Title: COMPOSITIONS COMPRISING NATURAL AGENTS FOR TREATMENT OF CANCER

(57) Abstract: Compositions are provided comprising five or more natural agents combined into one formulation, each natural agent possessing known anticancer activity, wherein the natural agents in the composition interact synergistically to enhance their anticancer activities. The compositions of this invention demonstrate *in vitro* inhibition of cancer cell proliferation and provide potential candidates for cancer therapies.

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INTERNATIONAL SEARCH REPORT

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PCT/US 01/12096

A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.G.L. HERTOG E.A.: "Intake and potentially anticarcinogenic flavonoids and their determinants in adults in the netherlands" NUTRITION AND CANCER, vol. 20, no. 1, 1993, pages 21-29, XP001073425 page 21 page 23 page 24	1,2,5, 12,15, 24-27, 30,37, 40,46, 49,50, 53,56, 63,66, 72,75
A	FR 2 100 669 A (AMERICAN GENERAL ENTREPRISES) 24 March 1972 (1972-03-24) claims 1,2,10 page 6, line 23-27 page 7, line 29-31	1,5,9, 11,21

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
16 April 2002	26/04/2002

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/12096

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 70949 A (M.C.Y. HENG) 30 November 2000 (2000-11-30) claims 1,60,62,67,70,81 page 24, line 11-14	1-5,9, 12-15, 18, 21-30, 34, 37-40, 43, 46-56, 60, 63-66, 69,72-75

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-75 relate to an extremely large number of possible compounds/products/methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those compounds described on page 42 of the present application, with due regard to the general idea underlying the present application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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